Collection of Blood Sample

Mostly we collect Venous blood sample to make most of investigation analysis that help in diagnosis.

Why we choose the Venous blood?

Because it is easy to obtain with minimum dangerous & containing all metabolic product which can easily measured.

- **Obtaining of venous blood sample**

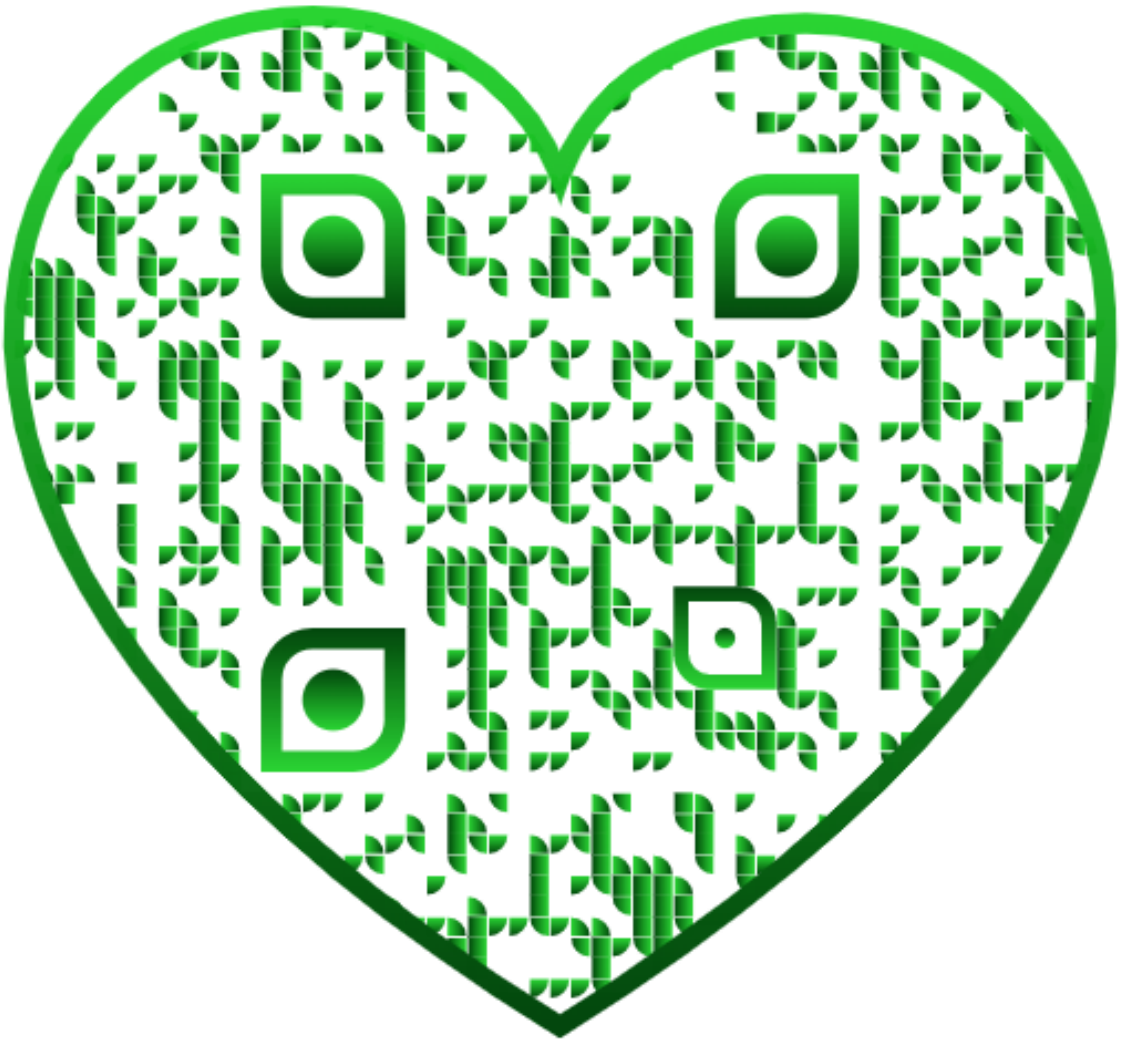
Equipment:

- Sterile Syringe
- Tourniquet
- Blood collection tubes
- Adhesive tape
- Sharps container

Venipuncture Procedure:

- Normally a venipuncture is performed with the patient sitting comfortably with good back support and the arm resting on a firm base. Sometimes the patient will request to lie down.
- It is important to select a vein carefully for blood collection. The antecubital fossa in front of the elbow joint is the best area where Median Cubital, Cephalic and Basilic veins pass through. The larger and fuller veins are used frequently.
  Veins on the underside of the wrist must not be used.
- Using the fingertip press firmly down on the skin and as you lift your finger you will feel the vein bounce back Move across the ante Cubital area like a warm rubber tube. Repeating the 'press down/release' motion until you locate a vein.
- Tourniquet is used to help distend the vein to allow a successful venipuncture. Apply the tourniquet 3 – 4 inches above the intended site.
  Do not put the tourniquet on too tightly or leave it on the patient longer than 1 minute.
- Once you have selected a suitable vein, Take aseptic precaution by using sterile material, & antiseptic alcohol swap or cotton.
- Swab the site with an Alcohol swab, Allow the skin to air dry. After the area is cleansed, it should not be touched or palpated again.
- Ask the patient to make a fist; avoid "pumping the fist." After you check the Syringe piston and needle, insert the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface. Hold the Syringe and Slowly drag the piston of syringe until you get the required amount of blood.
- Remove the tourniquet from the patient's arm before removing the needle.

Dr. M. Al-Khawi



- Remove the needle using a swift backward motion.
- Place gauze swab immediately on the puncture site and carefully re-cover the needle.
- Remove the needle from the Syringe and withdraw the sample into suitable tubes immediately depending on the sample which you need.
- label all test tube with the Lab reference number of patient.
- Dispose of all contaminated materials / supplies in Sharps container.

Areas to Avoid When Choosing a Vein

1. Scars from burns or surgery or obvious scarring from excessive needle use.
2. Haematoma or bruised areas.
3. Oedematous extremities will also alter test results due to the increased fluid accumulation.
4. The arm receiving IV infusion.

Complication

Immediate Complication: haematoma & syncope.
Late local complication: thrombosis of the vein.
Late general complication: transmission of blood born diseases.

To avoid formation of a hematoma, after you place the gauze swab on puncture site hold and apply adequate pressure for 1-2 minutes and tape a fresh piece of gauze or Band-Aid to the puncture site.

Capillary blood sample

For some tests we can use capillary blood sample that we can get from one of the following body sites by using special Lancet.

1) The lobe of the ear.
2) The palmer surface of the tip of the finger.
3) In infant even from the heel , or the great toe.

Arterial blood sample

Special skills and precautions are required for collecting of arterial blood sample.

Arterial blood sample mostly requested for analysis of blood gases.

Hemoglobin Concentration (Hb)

Weight of hemoglobin per a unit volume of blood (g/dl)

Cyanmethemoglobin Method (Drabkin's solution)

Potassium Ferricyanide, Potassium Cyanide, Sodium bicarbonate

SPECIFIC EQUIPMENT

1. Micropipette 20 ul
2. Pipette 5 ml
3. Spectrophotometer or colorimeter

SPECIMENS

1. Fresh blood
2. Anticoagulated blood

REAGENT (Drabkin's solution)

Potassium Ferricyanide, Potassium Cyanide, Sodium bicarbonate

PRINCIPLE

Hemoglobin + Ferricyanide $\longrightarrow$ Methemoglobin $\xrightarrow{\text{Cyanide ions}}$ Cyamethemoglobin

- Stable red compound
- Measured colorimetrically
- Its intensity is directly proportional to the amount of hemoglobin in the sample

PROCEDURE

1. Add 5 ml of the reagent into both the blank tube and test tubes
2. Use Micropipette to get 20µl of blood
3. Wipe off excess blood adhering to the pipette
4. Add 20µl of blood into the (test) tube
5. Mix well and allow to stand for 5 minutes
6. Use the blank tube to adjust the colorimeter to zero optical density at 540 nm wavelength.
7. Read the optical density Absorbance of the test
8. Calculate the Hb concentration by using standard curve, standard sample or given factor.

Hemoglobin Concentration (Hb)

CALCULATION

Hb Concentration (g/dl) = Sample Abs X Factor.
= 0. ---- x 37

- If you have standard sample with known concentration

$$\text{Hb Concentration (g/dl)} = \frac{\text{Sample Abs X Std. Conc}}{\text{Std. Abs.}}$$

N.R

Male: 14 – 18 g/dl
Female: 12 – 16 g/dl

Advantages

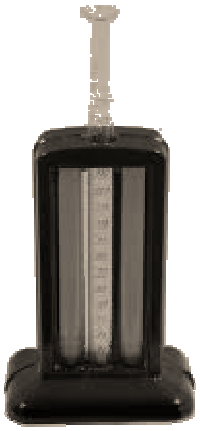
- Accurate
- Rapid
- Internationally adapted

Disadvantages

- Cyanide is toxic solution
- Lipemia may falsely increase the Hb values

Hemoglobin Concentration (Hb)

Sahli method (Acid hematin)



Sahli Apparatus

- Two Lateral Standard Tubes
- One Central Bigraduated Tube
- Sahli Pipette (20 µl)

PRINCIPLE

Hemoglobin + 0.1 N HCl ⟶ Acid hematin (brown color)

The resulting brown color is matched with brown glass standard

PROCEDURES

1. Place 0.1 N HCl into the central bigraduated tube till the mark 2 on the gram scale
2. Draw the blood into Sahli pipette exactly to the mark 20ul
3. Wipe off excess blood adhering to the pipette
4. Mix, let stand for 10 minutes
5. Add 0.1 N HCl or distilled water (drop by drop with mixing) until it matches the color of the standard tubes
6. Take the reading in g/dl scale

Hb conc. = ----- g/dl

N.R

Male: 14 – 18 g/dl
Female: 12 – 16 g/dl

Advantages

- Error may be 5-10% by careful technique

Disadvantages

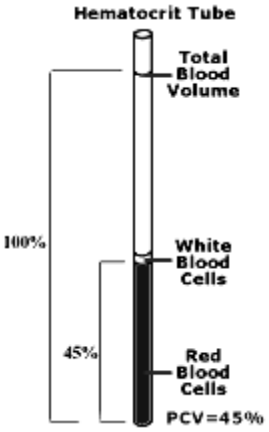
- Difficult color matching
- Color fading by time

Hematocrit (Hct) or Packed Cell Volume (PCV)

Ratio (in percent) of packed red cells in total whole blood (%)

SPECIFIC EQUIPMENT

1. Capillary tubes (1 x 75 mm) red & blue tip
2. Oily-clay sealer
3. Hematocrit centrifuge (12,000 rpm)
4. Hematocrit reader or Ruler



SPECIMENS

1. Fresh blood (red tip)
2. Anticoagulated blood (blue tip)

PROCEDURES

1. Mix the blood sample thoroughly.
2. Fill blood into capillary tubes for up to 4/5 of its length.
3. Seal bottom of the tube with oily-clay sealer.
4. Clean outside the tube with tissue paper nicely.
5. Place the tubes in to the rotor, adjust the bottom of the tube to close to the outer edge.
6. Close inner lid tightly, then close the outer lid.
7. Centrifuge for 5 minutes.
8. Open the lids after the rotor was completely stopped.
9. Read the value with Hct reader or ruler.

N.R

Male	=	40 - 52 %	(0.40 - 0.52)
Female	=	37 - 47 %	(0.37 - 0.47)

RBC Count (cont.)

Number of red blood cells per a unit volume

(x million / cu.mm) (x 10^{6} / cu.mm) (x 10^{6} / mm^{3}) (x 10^{6} / uL) (x 10^{12} / L)

SPECIFIC EQUIPMENT

1. Micropipette 10 - 20ul 2. Pipette 1 - 5 ml 3. Hemocytometer & cover slip (Counting chamber)

4. Microscope 5. Counter

REAGENT

Red cell diluting fluid Normal saline (Sodium Chloride 0.9% solution)

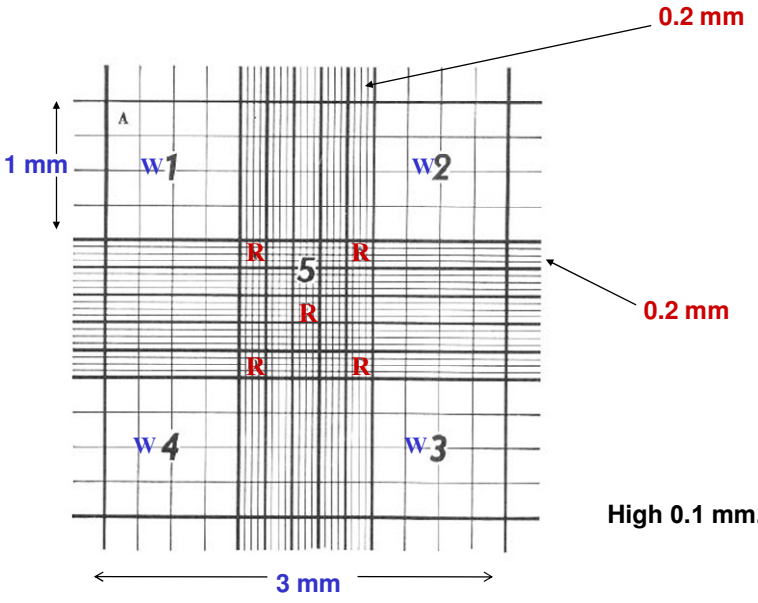
SPECIMENS

1. Fresh blood

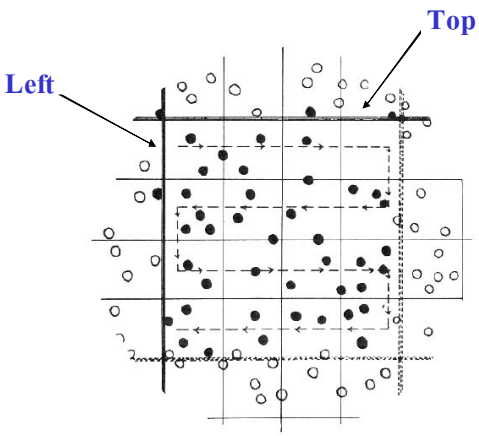
2. Anticoagulated blood

PROCEDURE

1. Mix the blood sample thoroughly.
2. Add 10 ul of blood to 2 ml of reagent or 20 ul to 4 ml (1:200)
3. Mix well for 1 min.
4. Fix the cover slip on the Hemocytometer (Counting chamber)
5. Charge the diluted blood into chamber counting area and allow RBC to set down
6. Count RBCs in 5 red squares of Hemocytometer under microscope (x40 objective)
7. Calculate the RBC count.



High 0.1 mm.



RBC Count (cont.)

CALCULATION

RBC Count = N (counted Number) x Volume Factor x Dilution Factor

Volume Factor = 1 / square volume (0.2 x 0.2 x 0.1) x (1 / No. of squares)

= (1 / 0.004) x (1 / 5)

= 250 / 5 = 50

Dilution Factor = 200

The final RBC count = N x 50 x 200

= N x 10000 (Cell/cu.mm.)

N.R

Male: 4.5 - 6.0 x 10^6 / cu.mm

Female: 3.8 - 5.2 x 10^6 / cu.mm

Blood Indices

Mean Corpuscular Volume (MCV) = Hct (%) x 10 / RBC (in millions / cu.mm.)

Normal range = 80 - 97 fL.

Mean Corpuscular Hemoglobin (MCH) = Hb (g/dL) x 10 / RBC (in million / cu.mm.)

Normal range = 27 - 31 (pg)

Mean Corpuscular Hemoglobin Concentration (MCHC) = Hb (g/dL) x 100 / Hct (%)

Normal range = 32 - 36 (% or g/dL)

WBC Count (cont.)

Number of white blood cells per a unit volume

(x 1000 / cu.mm) (x 10^3 / mm^3) (x 10^3 / uL) (x 10^9 / L)

SPECIFIC EQUIPMENT

1. Micropipette 20 ul 2. Pipette 0.1 - 1 ml 3. Hemocytometer & cover slip (Counting chamber)

4. Microscope 5. Counter

REAGENT

WBC Diluting fluids

2% Glacial acetic acid, Gentian violet

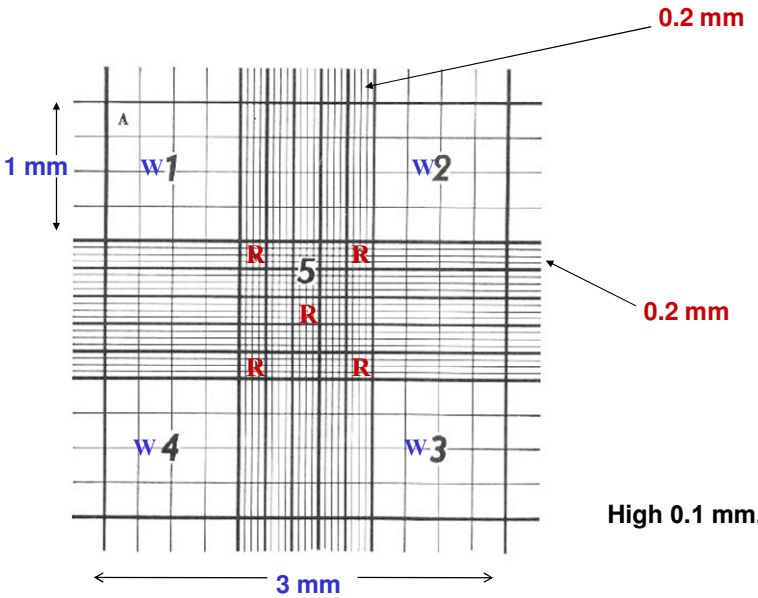
SPECIMENS

1. Fresh blood

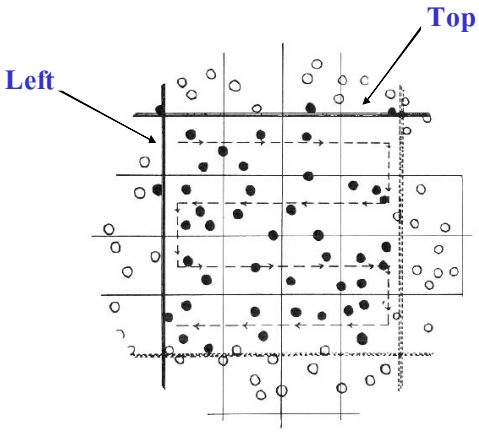
2. Anticoagulated blood

PROCEDURE

1. Mix the blood sample thoroughly.
2. Add 20 ul of blood to 0.4 ml of reagent (1:20)
3. Mix well for 1 min.
4. Fix the cover slip on the Hemocytometer (Counting chamber)
5. Charge the diluted blood into chamber counting area and allow WBC to set down
6. Count WBCs in 4 white squares of Hemocytometer under microscope (x10 objective)
7. Calculate the WBC count



High 0.1 mm.



WBC Count (cont.)

CALCULATION

WBC Count = N (Counted Number) x Volume Factor x Dilution Factor

Volume Factor = 1 / square volume (1 x 1 x 0.1) x (1 / No. of squares)

= (1 / 0.01) x (1 / 4)

= 10 / 4

= 2.5

Dilution Factor = 20

The final WBC count = N x 2.5 x 20

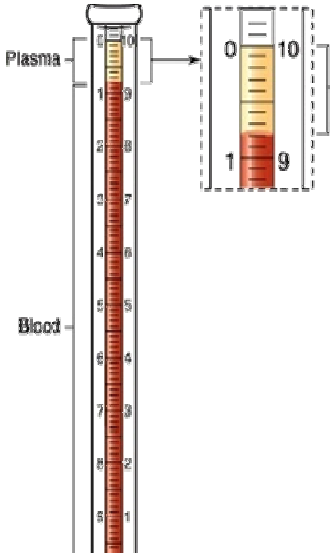
= N x 50 (Cell/cu.mm.)

N.R

4 - 11 x 10^3 / cu.mm

ERYTHROCYTE SEDIMENTATION RATE (ESR)

SPECIFIC EQUIPMENT



1. Westergren tubes (ESR tube)
2. Westergren rack
3. Timer 60 Min.

SPECIMEN

Diluted fresh anticoagulated blood (2 ml of blood to 0 .5 ml of sodium citrate or N.S)

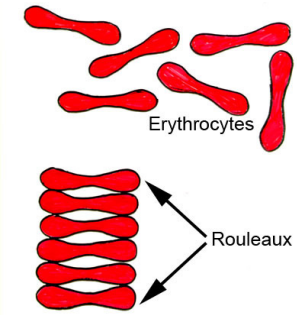
Blood should be at room temperature and should be no more than 2 hours old.

Hemolyzed specimens cannot be used.

PRINCIPLE

When anticoagulated whole blood is allowed to stand in a narrow vertical tube for a period of time, the RBCs settle out from the plasma. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour (mm/hr).

Various factors affect the ESR, such as RBC size and shape, plasma fibrinogen, and globulin levels.



The ESR is directly proportional to the RBC mass and inversely proportional to plasma viscosity.

In normal whole blood, RBCs do not form rouleaux.

In abnormal conditions when RBCs form rouleaux, the accumulated RBCs mass become greater, thus increasing the ESR (cells settle out faster).

PROCEDURE

1. Add well mixed blood to the plungeable vial.
2. Carefully insert the Westergren tube into the vial until blood reach to 0 level.
3. Place the tube in a vertical position on the rack.
4. Set timer for 1 hour.
5. After 1 hour has passed, read the distance of clear plasma present at the top of the column

N.R

Adult male	0-10 mm/hr
Adult Female	0-20 mm/hr

Blood Grouping ABO & Rh Typing

SPECIFIC EQUIPMENT

1. Slides or test tubes.
2. Wooden applicator.

SPECIMEN

- Fresh blood or Anticoagulated whole blood (Slide Method).
- Washed 5% RBCs suspension (Tube Method).

REAGENT

- Anti - A, Anti - B and Anti - D (Anti-sera).
- Anti-AB antibodies (optional)

PRINCIPLE

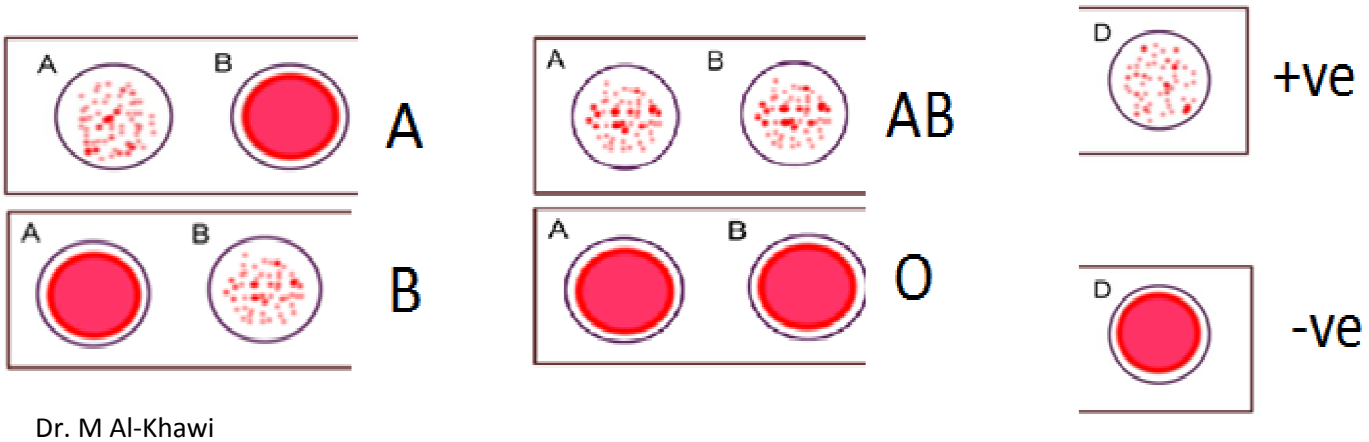
When red cells are mixed with Anti-sera (soluble antibody), agglutination will occur on the slides when RBCs contain the related antigen.

No agglutination will occur when the red cells do not contain the related antigen.

PROCEDURE

1. Label the slide or tube (A, B and D)
2. Place one drop of antibody A (blue) bottle on A section of the slide or in the tube labeled A.
3. Place one drop of antibody B (yellow) bottle on B section of the slide or in the tube labeled B.
4. Place one drop of antibody D (colorless) bottle on D section of the slide or in the tube labeled D.
5. Place one drop of blood on each antibody section of slid (Slide Method), or add one or two drops of RBCs suspension into each tube (Tube Method).
6. Carefully mix blood and Anti-sera with a separate applicator stick.
7. Rotate slide slowly for one minute, then observe for the agglutination.

INTERPRETATION OF THE RESULTS:



Measurement of Bleeding Time

Duke Method

EQUIPMENT

Alcohol wetted, cotton swab.

Sterile blood lancets

Filter-paper

Stopwatch

PRINCIPLE

A small cut is made with a sterile lancet in the earlobe or fingertip, Blood flows from the puncture and the time it takes form lancing till the bleeding stop is measured.

PROCEDURE

1. Gently clean the earlobe or fingertip with Alcohol swab and Allow to dry.
2. Use the lancet to make puncture on the earlobe or fingertip, start the stopwatch and observe the blood flow.
3. After 30 seconds collect the first drop of blood by the corner of filter-paper, do not touch the skin with the paper.
4. Repeat step 3 every 30 seconds until blood flow is stopped.
5. Stop the stopwatch and record the bleeding time.

N.R 1 – 5 Minutes

Measurement of Bleeding Time

Ivy Method

EQUIPMENT

Sphygmomanometer

Alcohol wetted, cotton swab

Sterile blood lancets

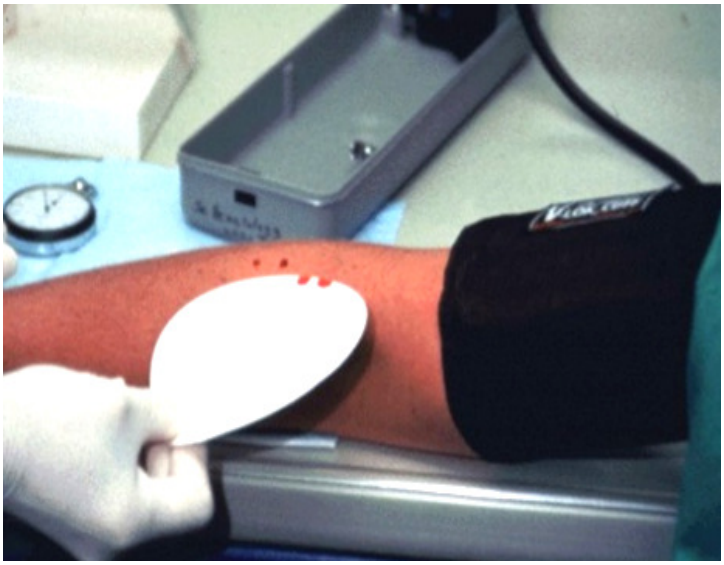
Filter-paper

Stopwatch

PRINCIPLE

The blood pressure cuff is placed on the upper arm and inflated to 40 mmHg; 2 or 3 small cuts is made with a sterile lancet on the underside of the forearm, Blood flows from the punctures and the average of bleeding time in all punctures is measured.

PROCEDURE



1. Place the cuff of blood pressure on the upper arm and inflated to 40 mmHg.
2. Locate the puncture site on the lower arm and clean it with Alcohol swab and Allow to dry.
3. Use the lancet to make 2-3 puncture, start the stopwatch and observe the blood flow.
   Cuts should be deep enough to cause slight bleeding and avoid vein puncture.
4. After 30 seconds collect the first drop of blood from each puncture by the corner of filter-paper, do not touch the skin with the paper.
5. Repeat step 4 every 30 seconds until blood flow is stopped.
6. Record the bleeding time of each puncture and calculate the average.

N.R 2 – 6 Minutes

Measurement of Clotting Time

PRINCIPLE

The coagulation time of whole blood is the time required for a blood sample to clot under certain specified conditions.

- Capillary Tube Method

EQUIPMENT

Alcohol wetted cotton swab - Sterile blood lancets - Nonheparinized capillary tubes - Stopwatch

PROCEDURE

1. Clean the fingertip with Alcohol swab and Allow to dry.
2. Use the lancet to make puncture on the fingertip and start the stopwatch.
3. Fill one or more capillary tubes with blood by lowering the end of fitted capillary around ¾th of its length.
4. After about two minutes start break a small piece of capillary.
5. Repeat breaking at regular time intervals (30 seconds), till fibrin thread appears at the broken end of capillary tube.
6. Stop the stopwatch and record time between pricking finger and first appearance of fibrin thread at the broken ends of capillary tube.

N.R 3 – 6 Minutes

- Slide or Drop Method

EQUIPMENT

- Alcohol wetted, cotton swab - Sterile blood lancets - Glass slide - Stopwatch

PROCEDURE

1. Clean the fingertip with Alcohol swab and Allow to dry.
2. Use the lancet to make puncture on the fingertip and start the stopwatch.
3. Place a large drop of blood on a clean and dry glass slide.
4. Draw a lancet tip through the blood drop and move it out every 30 seconds until you note the fibrin thread adhere to the lancet tip.
5. Stop the stopwatch and record time between pricking finger and appearance of fibrin thread.

N.R 2 – 5 Minutes